

The University of Chicago

THYROID-FORMING POTENCIES OF
THE EARLY CHICK BLASTODERM

A DISSERTATION SUBMITTED TO THE
GRADUATE FACULTY IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOÖLOGY

By
DOROTHEA RUDNICK

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Reprinted from
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TWO TEXT FIGURES AND TWO PLATES (EIGHT FIGURES)

INTRODUCTORY

The present analysis concerns itself primarily with the mediolateral localization of thyroid-forming potencies in the chick blastoderm before there is morphological indication of a thyroid primordium. The experimental method used consists in isolating certain definite portions of the early chick blastoderm and in testing the developmental capacities of these areas by growing them as grafts on the vascularized chorio-allantois of older chick embryos.

A brief survey of the genesis of the particular problem presented by the thyroid might be not amiss here. For the past several years, a research program conducted in this laboratory by Dr. B. H. Willier has been in part directed toward the analysis of the potentialities of the chick blastoderm at stages of the definitive primitive streak, head process, and early somites, under the conditions of the chorio-allantoic graft. Examination of grafts of the whole area pellucida of blastoderms of these young stages revealed the frequent presence of thyroid, in company with many other tissues (Willier and Rawles, '31 a). These grafts were of especial interest by reason of demonstrating a coincidence of development of heart and liver, and a frequent doubling of these organs.¹ In order better to study this situation, a series of experiments was performed in which the area pel-

¹ For special analysis see Willier and Rawles, '31 b.

lucida of blastoderms of the stages already mentioned was split in the median line, and the right and left halves grafted separately. This material, unpublished at present except in abstract form (Willier, etc., '31), besides showing that both halves of the blastoderm are capable of producing both heart and liver, indicated also a similar distribution for thyroid potentialities. At this time my own work with the morphologically patent thyroid anlage had led me to look for thyroid in preprimordial stages; the foregoing interesting discovery suggested my beginning the set of experiments on the primitive-streak and head-process stages which supply most of the data for this paper. I have had the pleasure of examining some of the grafts of right and left halves of blastoderms in the head-process stage, obtained by Doctor Willier and Miss Rawles. These, in connection with some of my own material, have been the basis of an incomplete preliminary report ('30);² I shall use them to a certain extent in my section on the head-process stage.

METHODS

The method of the chorio-allantoic graft, as practiced in this laboratory, has been described by Willier ('24). Briefly, the procedure is as follows.

a. The host is of about nine days' incubation. It is candled; the junction of two large blood vessels on the chorio-allantoic membrane may thus be marked. At this place, a window is made in the shell with a sharpened hacksaw blade.

b. The donor tissue is dissected, or otherwise prepared for transplantation, in a Petri dish containing warm sterile 0.9 per cent NaCl solution, under a binocular dissecting microscope. In the present experiments, this preparation involves freeing the blastoderm from the yolk and from the vitelline membrane and spreading it flat in the dissecting dish by means of repeated flooding with salt solution from a pipette.

² Part of this investigation was carried out during an appointment as research assistant to Doctor Willier, under a grant to the University of Chicago from the Rockefeller Foundation.

The stage of development is ascertained with exactness; the cuts to be made are then marked out lightly with a sharp Bowman or Knapp knife-needle; by this means, the pieces to be isolated may be seen in their true relation to one another before the final cuts are made. The tendency of the cut blastoderm to curl and to pull apart makes such observations possible only before any complete cut is made.

c. The piece to be transplanted having been isolated from the rest of the blastoderm, the window in the host shell is removed, and the underlying shell membrane torn for a short distance. The edges of this tear are held apart with forceps, and the donor tissue is placed on the blood vessels of the chorio-allantoic membrane beneath, the transfer being accomplished by means of a needle or a fine pipette. This done, the shell membrane is allowed to close; the window in the shell is replaced and sealed with paraffin; the egg set window down on a watch-glass, and returned to the incubator. It is kept in this position for twelve hours, then is removed from the watch-glass and is allowed to lie free on the tray with the other eggs.

d. When the host is eighteen days old, it is opened, and the graft removed, fixed, and sectioned.

In these experiments, eggs from one single-comb White Leghorn strain were used almost exclusively. These were supplied by a reliable poultry breeder. Donor eggs for definitive primitive-streak stages were incubated for twelve to fourteen hours; head processes were obtained up to twenty-four hours. There is great variability in the early hours of incubation; hence the developmental stage rather than the time of incubation is of importance here. Hosts were incubated for eight to ten days before the operation. The incubator was kept at 38 to 39°C.; the sand tray was kept moist, and the eggs in the trays rolled every day.

For histological examination of the grafts, Bouin was used as a fixing agent; serial sections were made at 8 μ , stained in Heidenhain's iron haematoxylin, both long and short methods being used. Counterstaining was done with eosin.

For the original outlining of this problem and for aid and comfort during its progress, as well as for his generosity in placing his own material at my disposal, I offer my most appreciative thanks to Doctor Willier. Miss Mary Rawles, of this laboratory, also, has been most kind; it is a pleasure to acknowledge indebtedness to her.

ANALYSIS OF GRAFTS

a. Results from grafts of thyroid primordium

As is so frequently the case in embryological studies, it is illuminating to reverse rather than to follow the order of development: to use results from older, more differentiated material in interpreting those from younger stages. Accordingly, grafts from three-day chick embryos will first be summarily considered.

In the seventy-two-hour chick (c. 35 somites), as is well known, the primordia in the pharyngeal region are clearly laid out. In particular, the thyroid, which has been present as a distinguishable area in the floor of the foregut at the level of the second pair of branchial pouches since the 12-somite stage (Lillie, '19), is at this time a spherical median diverticulum, in the process of being constricted off.

Isolations of the thyroid anlage at this time are usually not perfect; fragments of the gut to which it is attached, and of mesenchyme adherent to its surface, are apt to be included in the transplant. One case, however, was obtained in which no structure other than thyroid differentiated (13-11). Other cases contained a trace of gut or cartilage. Transplants of a larger portion of the branchial region, in which the thyroid anlage was included, also showed differentiation of this gland. The single case where isolation was complete is adequate to prove that the thyroid primordium is a self-differentiating system at this stage.

Graft 13-11 is interesting, moreover, in that the thyroid found therein occurs as two distinct masses. This might be interpreted as demonstrating that not only the capacity for histological self-differentiation, but also the capacity to di-

vide into two bodies as in normal morphogenesis, is intrinsic in the thyroid anlage at seventy-two hours. Such a conclusion is, however, unwarranted. Grafts in which other tissues as well as thyroid are present, contain from one to three thyroid bodies. Clearly, the single thyroid primordium is capable of division; but the products of this division are not limited to two.

The histology of thyroid material in these grafts will be discussed in section *d*.

b. Potential thyroid: localization and properties

1. *Head-process stage.* If the pellucid area of a blastoderm in the head-process stage be examined, certain reference points in its topography may be noted. First, there is the primitive streak, which marks the median line, extending forward from near the posterior margin, and terminating anteriorly in the node of Hensen, in the center of which is seen the primitive pit. Anterior from Hensen's node extends the head process or notochord. In early head-process stages, this structure shows through the overlying ectoderm as a not too well-defined longitudinal thickening, broader at its anterior than at its posterior end. Later, in stages which are designated in the table, for convenience, as HP (in contrast with early head process or EHP), the notochord appears as an evenly thin, well-marked line, longer than in previous stages. The third phase in development before the appearance of somites is distinguished by the formation of the head fold at the anterior end of the head process, as well as by more extensive regression of the node. This stage will be indicated in the table as HF. With the most advanced head-process stages, where the medullary folds become distinct and the vertebral plate well marked, I have not dealt separately.

The head-process material lent me by Doctor Willier, to which reference has been made above, consists of five pairs of grafts, obtained in the following manner: a transverse cut was made in the area pellucida anterior to the primitive

knot. The material in front of this cut was divided, by a cut splitting the head process, into right and left halves. These pieces were grafted separately. In five cases well-grown grafts were obtained from both right and left half of a single donor.

The presence of thyroid in both halves in three of these five pairs is convincing proof that thyroid-forming potentialities are bilaterally located at this stage. It is also of interest that, while all five grafts from the left half contain thyroid, only three of the right halves give positive results, although thyroid may possibly be present in a fourth case.

Clearly, the next question to be answered was this: Is prospective thyroid material localized with reference to the transverse axis of the blastoderm? if so, how? if not, is thyroid formation the function of a level along the antero-posterior axis, or is it the result of a specific induction on the part of some other tissue functioning in the graft? Accordingly, the following experiments were performed.

Grafts were made in this manner: longitudinal cuts parallel to the head process on either side of the node and well outside the limits of the node divided the anterior end of the pellucid area into three pieces: right, median, and left. The transverse cut varied somewhat: in early stages, where the head process was very short, this cut was made posterior to the node; in most of the cases, however, the node level was not included. Figure 1 shows these cuts in diagrammatic fashion on a medium or HP stage.

The percentage of successful grafts from pieces of early blastoderms is often distressingly low. Out of a total of thirty-nine transplants of left pieces in which the host remained alive until the eighteenth day, ten grafts suitable for recording have been obtained (26 per cent). Of forty-nine possible grafts of right pieces, eleven are recorded (23 per cent). Nineteen out of fifty-four possible median pieces are tabulated (35 per cent).

Grafts from lateral pieces are in general less well grown than those from median strips. They are smaller, contain

fewer tissues, and less luxuriant growth of those tissues than do grafts from median pieces. Brain and lymph vesicles, in conjunction with masses of solid tissue, are the most conspicuous macroscopic features of both types of graft.

Table 1 presents a complete account of tissues found in these grafts, grouped according to type of graft, and listed according to age of donor. Individual variability in grafts makes this statistical treatment necessary. It must also be understood that this variability prevents our giving weight to negative cases short of 100 per cent; our interpretations



Figure 1

must be made on the basis of positive structures identified (indicated in the table by +). Before considering the implications of the distribution of thyroid, certain orienting observations may be useful.

In the first place, certain tissues are common to all types of graft, whether lateral or median. These tissues include the generalized ones found in almost all types of graft from early stages: skin, gut, and somite derivatives; as well as brain, a structure whose primordium is extensive laterally.

Secondly, however, we observe cases where structures are found exclusively in one type or the other of graft. Such cases are those of the chorda, which is confined to median

TAB 1
Structures in grafts

PIECE	SERIAL NO.	STAGE	HEART	LIVER	THYROID	ORAL CAVITY	PHARYNX	TRACHEA	LUNG	SPINAL CORD	BRAIN	GANGLIA
R	45-16	EHP	+	+	...		...	+	+	...	+	+
	69-4	EHP	+	+	+		...	...	...	...	...	+
	74-1	EHP	+	+	...		...	...	...	...	...	...
	63-1	HP	+	++	+		+	...	...	...	+	+
	63-13	HP	+	+	...		...	...	...	...	+	...
	68-7	HP	+	+	+		+	...	...	...	+	+
	43-8	HF	+	...	...		...	...	...	...	...	...
	52-7	HF	+	...	...		...	...	...	...	+	...
	59-13-4	HF	...	...	...		...	...	...	...	+	...
	65-6	HF	+	+	...		...	...	...	...	...	...
	75-12-5	HF	+	+	...		...	...	...	...	...	...
L	67-4	EHP	...	...	...	...	...	...	...	...	+	...
	74-9	EHP	+	...	...		...	...	...	...	+	...
	38-15	HP	...	...	...		...	...	...	...	+	...
	51-13-1	HP	+	+	+	+	+	+	...	...	+	+
	41-3	HF	+	+	+		+	...	...	...	+	+
	45-1-2	HF	...	+	+	+	...	...	...	...	+	+
	45-17	HF	+	++	...		...	...	...	...	+	+
	46-18	HF	++	...	...		...	...	...	...	+	...
	49-3-3	HF	+	...	+		+	...	...	...	+	+
	57-3	HF	+	...	...		...	...	...	...	...	...
M	45-15	EHP	...	...	...		...	...	...	...	+	...
	51-11	EHP	...	...	...		...	...	...	...	+	...
	60-11	EHP	...	...	?		?	...	...	...	+	+
	63-8	EHP	...	...	...		...	+	...	...	+	+
	66-6	EHP	...	...	+	+	...	...	...	...	+	+
	68-4	EHP	...	...	...		...	...	...	...	+	...
	74-7	EHP	...	+	...		...	+	...	...	...	...
	75-15	EHP	...	...	...		...	...	...	...	+	+
	90-2	EHP	...	...	...	+	...	...	+	...	+	+
	38-20	HP	...	+	+	+	+	...	...	...	+	+
	51-14-1	HP	...	...	...		...	...	...	...	+	...
	38-2	HF	...	...	...		...	...	...	...	+	...
	39-12	HF	...	...	...		...	...	+	...	+	...
	45-2-2	HF	...	...	...		...	...	...	...	+	...
	49-2-3	HF	...	...	...		...	...	...	...	+	...
	52-14	HF	...	...	...		...	...	...	...	+	...
	59-14-4	HF	...	...	...		...	...	...	...	+	...
	61-5	HF	...	...	...		...	...	...	...	+	...
	75-13-5	HF	...	...	+	+	...	...	...	...	+	+

pieces, and heart, which is found only laterally. This type of result indicates certainly a strict localization of prospective material with reference to the transverse axis: dorsal structures restricted to the median portion; ventral structures double and laterally placed. But in these cases we are dealing with actual formed primordia, about the localization and segregation of which there is no question.

Lastly, we come to the more perplexing cases of median ventral structures, such as thyroid and liver, which are nevertheless found in median pieces as well as in lateral ones. This discussion will concern itself only with the thyroid; although liver parallels the distribution of thyroid so closely that the words might be interchanged throughout this description without violence to its veracity.

Of the occurrence of thyroid, we may say that the table demonstrates beyond question the presence of this structure in lateral pieces (in 27 per cent of cases on the right, and 40 per cent on the left): that is to say, in a position corresponding to the future location of the thyroid primordium. The capacity of certain median pieces (21 per cent) to yield thyroid is, then, the result not to be expected a priori, requiring interpretation which I hasten to supply. It might be well to begin by inspecting the cases available where two contiguous transplants from one donor have yielded grafts. These cases are marked by a figure in the column following that showing serial numbers in the table (e.g., grafts 51-13 and 51-14, marked case 1, came from the same donor).

The story is clear-cut in so far as it concerns the left side. Where thyroid occurs in the left lateral piece, it does not occur in the median (cases 1, 2, 3). For the right side, nothing decisive is found. The fifth case, that of grafts 75-12 and 75-13, appears to be a direct contradiction of the results for the left side; but this case was an exceptional type of graft. My operating notes state that the donor blastoderm here was in an advanced head-fold stage, and that the lateral cuts were made, not next to the node, but at the edges of the segmental plate, which was obvious. The median piece was thus very

wide indeed; so that the result cannot be compared with the preceding material, although it is of interest in showing the probable lateral limit of prospective thyroid at this stage.

Further examining the table, we may note that in no case is thyroid found in a median piece in the head-fold stage, if we except the case 75-13, explained above. The blastoderm undergoes a tremendous amount of growth during the head-process stage; it therefore follows that a median piece cut with reference to the node will in a head-fold stage contain relatively less material than a similar piece from an earlier stage.

This consideration suggests the very plausible explanation that the thyroid obtained in certain median grafts represents part or all of laterally located segregates³ included in a relatively wide median strip. On the other hand, we cannot rule out the hypothesis that prospective thyroid, before the head-fold stage at least, is present all across the blastoderm, at a given level, say, of the anteroposterior axis. Since such an area, being more extensive than the future primordium and thus containing portions having prospective potency other than that of thyroid, could not be a segregate, the implication would be that the time of segregation has been located; i.e., coincident with formation of the head fold. There is, as well, the third hypothetical possibility that thyroid tissue found in these grafts has arisen *de novo* from indifferent tissue, as a result of a specific induction.

There are, I believe, definite indications that thyroid in the grafts has arisen from tissue that was at the time of operation not indifferent. This point will be discussed more fully in connection with the primitive-streak material; but it may be noted here that only one structure in the grafts fulfills the basic requirement for an agent inducing thyroid after the transplantation had been effected: that of being found

³ The term *segregate* I employ in the sense defined by F. R. Lillie ('29) viz., an area of specific potency, capable of self-differentiation in a neutral medium. Since a chorio-allantoic graft containing many tissues and organs does not offer a strict test of self-differentiation, evidence for segregation in this type of work must necessarily be indirect.

consistently in juxtaposition to thyroid. This structure is gut, variously identified as oral cavity or pharynx. Since such gut is not always of the same histological character (section *d*) and, moreover, may be very weakly developed, it seems to possess neither the specificity nor the activity requisite to a tissue capable of producing a specific differentiation in contiguous indifferent material, in the graft.

Aside from gut, the only constant component of grafts containing thyroid is the ganglion cell. Such cells are frequently, though not necessarily, in contact with thyroid. There can be no question of an induction here; but the possibility suggests itself that the presence of nervous tissue may be a condition for differentiation of thyroid in grafts from this stage.

2. Definitive primitive-streak stage. It became clear in the course of certain preliminary experiments with the definitive primitive streak that not only must the median piece be made as narrow as possible in order to resolve the question of whether prospective thyroid were present laterally, but that measurements of the pieces cut were necessary. It did not at this time, however, appear desirable to dissect the node itself. In the experiments to be reported, then, the following procedure was employed:

The chick blastoderm at the stage of the definitive primitive streak, it will be recalled, presents two striking landmarks: the primitive streak and the well-developed primitive knot. The pit, at the center of the knot, was chosen as the main point of reference (fig. 2, *P*). In performing the operation, the cuts *AA'*, *BB'*, *CC'* were marked out, *AA'* being a transverse cut well behind the node; and the two longitudinal cuts *BB'* and *CC'* being made, as nearly as possible, just at the edges of the node. While the blastoderm was in this state, measurement of six distances was made with the ocular micrometer. These measurements were: the transverse distance from the pit to each lateral edge of the area pellucida (*PM*, *PN*); the transverse distance from the pit to the cuts *BB'* and *CC'* (*PM'*, *PN'*); the length of the primitive streak

(PQ); and the distance from the pit to cut AA' (PQ'). The cuts were then completed, and the right, median, left, and posterior pieces grafted separately.

The posterior part of the blastoderm was grafted in this series partly as a check and partly for purposes irrelevant to the questions at hand. No graft of such a piece has been found to contain thyroid; hence no further mention will be made here of these grafts.

Grafts of lateral as well as of median pieces tend in these experiments to be large, well-grown, and well-differentiated.

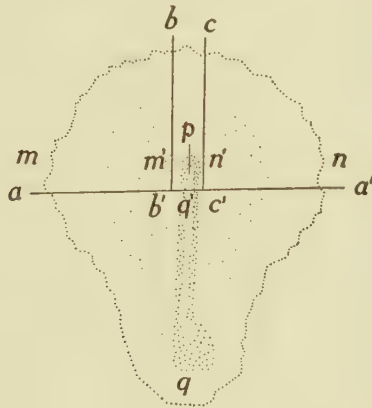


Figure 2

The median grafts, on the whole, are larger, and more frequently obtained, the yield being 36 per cent positive cases (thirty-two out of ninety possibilities) as contrasted with 25 per cent and 28 per cent, respectively, for approximately the same number of transplants of right and left pieces. These figures include as positive even questionable grafts; for examination and tabulation, it has been possible to use eleven grafts of right pieces (13 per cent), twelve of left (15 per cent); twenty median grafts of good size have been selected for histological study.

Table 2 records the structures found in the grafts described above, together with the distances PM' and PN' in milli-

TABLE
Structures in grafts from

PIECE	SERIAL NO.	STAGE	PM'	PN'	HEART	LIVER	THYROID	ORAL CAVITY	PHARYNX	TRACHEA	LUNG	SPINAL CORD
R	77- 2-A-1	DPS—		.15	...	++	...	+	...	...	...	...
	77- 5-A	DPS		.21	...	+	...	+	...	...	...	?
	78- 2-A	DPS		.21	+	+	+	+	...	+	...	...
	85- 5-A	DPS		.16	+	+	...	...	...	...	...	...
	86- 9-A-2	DPS		.20	...	...	...	...	...	...	...	...
	97- 1-A	DPS		.20	...	...	...	+	...	...	...	...
	99- 4-A	DPS		.20	+	+	...	...	...	...	...	...
	108-10-A-6	DPS		.14	...	...	...	...	...	...	...	...
	79- 3-A	DPS+		.15	...	...	...	+	...	...	...	...
	105- 1-A-5	DPS+		.20	+	+	...	...	...	...	...	...
	96- 3-A	DPS+		.12	+	+	...	...	...	...	...	...
L	88- 3-C-3	DPS—	.12		+	...	+	+	...	...	...	...
	96- 2-C	DPS—	.16		+	+	...	+	...	...	...	...
	100- 5-C	DPS—	.12		...	...	...	...	...	...	...	...
	77- 1-C	DPS	.17		...	...	...	...	...	...	...	...
	78- 4-C	DPS	.18		...	...	...	...	...	...	...	...
	86- 9-C-2	DPS	.16		+	++	+	+	+	+	...	...
	93- 2-C	DPS	.14		+	...	...	+	...	...	...	...
	96- 4-C-4	DPS	.12		+	+	...	...	...	+	+	...
	96- 5-C	DPS	.10		...	...	...	+	...	...	...	...
	108-10-C-6	DPS	.16		+	...	...	...	...	...	...	...
	86- 6-C	DPS+	.14		+	+	...	+	...	...	...	...
	94- 2-C	DPS+	.14		+	++	+	+	...	...	...	...
M	77- 2-B-1	DPS—	.18	.15	...	+	...	+	...	+	...	...
	82- 1-B	DPS—	.11	.11	++	++	++	+	...	++	...	...
	88- 3-B-3	DPS—	.12	.10	...	...	...	...	...	...	...	...
	94- 3-B	DPS—	.18	.14	+	+	+	+	...	+	...	...
	103- 2-B	DPS—	.10	.18	+	...	+	+	+	+	...	...
	107- 1-B	DPS—	.12	.20	++	+	+	+	...	++	+	...
	84- 5-B	DPS	.16	.20	++	++	+	+	...	...	+	...
	86- 9-B-2	DPS	.16	.20	...	...	...	...	...	+	...	...
	93- 3-B	DPS	.12	.20	+	+	+	+	...	...	...	+
	93- 4-B	DPS	.12	.18	...	...	+	+	+	++	...	...
	95- 2-B	DPS	.24	.16	...	+	...	...	...	...	...	...
	96- 4-B-4	DPS	.12	.14	...	+	+	...	...	...	...	?
	97- 2-B	DPS	.16	.12	...	...	...	...	...	++	+	...
	97- 5-B	DPS	.20	.20	+	+	+	+	...	...	...	...
	100- 4-B	DPS	.16	.16	...	...	...	+	...	+	...	...
	102- 2-B	DPS	.12	.16	...	...	...	+	...	+	...	...
	108-10-B-6	DPS	.16	.14	...	+	+	...	+	+	...	+
	78- 6-B	DPS+	.15	.18	?	+	...	...	+	+	...	...
	92- 3-B	DPS+	.20	.16	++	...	+	+	+	...	+	...
	105- 1-B-5	DPS+	.16	.20	...	...	...	+	...	+	...	+

definitive primitive-streak stage[illegible]

meters. It will be seen at once that, while typical ventral structures, such as heart, liver, and thyroid, are indeed found in lateral pieces, they are also found with great frequency in median grafts: the situation appears to be an exaggeration of that observed in the earlier head-process grafts. For thyroid, the percentage of positive cases is 9 for right pieces; 25 for left; 55 for median.

Consideration of the cases where two or three grafts were obtained from one donor will serve to illustrate the point. For this purpose, such cases are indicated as previously in table 1. The two cases where all three grafts from one donor are available (86-9 and 108-10) show, on one hand, thyroid appearing in the left graft, not in the median or right; on the other hand, thyroid in the median but not in the lateral pieces. Add to this case 88-3 where thyroid occurs in the left piece, but not in the median; case 96-4 where it is found in the median piece, but not in the left.

Some slight indication of the extent of prospective thyroid may be obtained by considering the measurements made at the time of transplantation. Transverse distances from the primitive pit to the longitudinal cuts (fig. 2, PM' , PN') are given in millimeters in table 2. It may be well to state here that the transverse extent of the node (measured on ten fixed and stained preparations) ranges from 0.06 mm. to 0.12 mm. on the right side of the pit; from 0.08 mm. to 0.12 mm. on the left. Since the width of the area pellucida in blastoderms of this stage varies considerably, it would be preferable to express distances rather by the ratios $\frac{PM'}{PM}$ and $\frac{PN'}{PN}$; however, the gain in accuracy would be too slight to justify such elaboration on figures that are by the nature of the case only approximate.

In the graft material, thyroid is found on the right side at least as far laterally as 0.21 mm. (graft 78-2-A); at least as near the median line as 0.11 mm. (82-1-B). On the left side the extent is laterally as far as 0.14 mm. (86-9-C); medially as close as 0.10 mm. (82-1-B, 103-2-B). These values, it must be understood, show absolutely minimum extent, as the

data read. The figures are perhaps worth little; it must be recalled that the cuts were performed freehand on delicate and elastic material, and were probably not always perfectly parallel; as they stand, however, they indicate a rather surprisingly extensive potential thyroid area.

In this material, again, it is impossible to decide whether we are dealing with one potential thyroid area, extending broadly across the blastoderm, or with two localized areas. The fact that in grafts of median pieces of the primitive-streak stage, heart is frequently obtained, makes it clear that, although this strip was cut as narrow as was possible without encroaching on the node itself, it yet was wide enough to include all of the prospective ventral organs.

A relevant question is whether prospective thyroid in the stage of the definitive primitive streak is segregated. Again, similarly to the head-process grafts, thyroid is found to appear independently of structures other than gut, cartilage, and nervous tissue. The latter cannot possibly be a specific inducing agent for thyroid, since the two are only occasionally found contiguous. The same is true of cartilage, a tissue whose general nature also makes it the most unlikely of candidates. For reasons mentioned previously, it seems improbable that gut, either, can be regarded as inducing thyroid, though the consistent association of these two structures makes such an interpretation not impossible. If induction is a factor effective in the determination of thyroid, an agent had better, it might be suggested, be sought in the overlying germ layers rather than in the entoderm itself (see Waddington, '30); and at stages earlier than those hitherto used.

In these grafts, as well as in the head-process material, there is a decided tendency of thyroid, heart, and liver, with associated gut and ganglion cells, to be found in the same region of the graft (figs. 5 and 7). The association of thyroid with heart and liver is not a necessary one, as is proved by its independent occurrence in several cases. Nevertheless, the fact that this association occurs typically speaks strongly for a localized, and therefore to some degree specific, area of

prospective thyroid which tends to maintain its topographical relations to similarly localized neighboring areas.

A further point, which supports the interpretation that thyroid at the time of transplantation possesses at least a degree of autonomy, is that in these grafts thyroid frequently appears, not as a single mass, but in several lumps having no direct connection one with another. This fact does not appear in the tables, since, being anxious to find possible cases of genuine duplication, I have never recorded more than one thyroid present unless separate masses appear in quite different parts of the graft, in connection with independent gut tubes. Typically, thyroid in a well-grown graft of this series appears as one or two or more lobes, separate yet connected with one oral cavity or gut complex. Such an arrangement, it would seem, indicates the breaking up of an already segregated area, by the mechanical conditions of the graft, rather than the establishment *de novo*, in several different localities, of the precise conditions required for an epigenetic evocation of thyroid.

The measurements performed, by indicating a rather disproportionately large area capable of differentiating as thyroid, suggest rather interestingly a thyroid field in the definitive primitive-streak blastoderm: that is to say, a center or centers of differentiation with relatively large surrounding area showing the same potencies, yet in capacity diminishing as distance from the center increases. The implication is that thyroid, present in the definitive primitive-streak stage as a broad field across the area *pellucida*, whether with a single center or with double bilaterally located centers, during the head-process stage becomes a localized bilateral system, as is indicated by the decrease and final cessation of capacity of median pieces to yield thyroid. The frequent multiplication of masses of thyroid may be thought indicative of an original potential thyroid area of some size. This is an interpretation on which one cannot insist, in view of the slight data on which it is based.

The evidence at hand, one might say in summary, indicates that by the stage of the head fold there are two prospective thyroid areas, situated ahead of the node, symmetrically located with reference to the median line, removed from it by a distance greater than the lateral extent of the node, but lying within the limits of the vertebral plate. That this situation holds true also in earlier stages, with the qualification that the prospective thyroid areas are closer together, is very possible. The alternative is that a single field is present. What has been demonstrated is that, at an early stage, before the formation of the gut, the regions that have the prospective value of thyroid, have under the conditions of the chorio-allantoic graft, the capacity to yield specifically differentiated thyroid, independently of direct induction, though not necessarily independently of other correlative factors. At three days the thyroid, a well-marked primordium, is a true segregate.

c. The problem of asymmetry

As may be seen from the most casual examination of the tables here presented, thyroid is obtained from the right side less frequently than from the left. The exact figures are these: in my head-process material, three cases out of eleven right grafts contain thyroid (27 per cent); on the left, four cases out of ten, or 40 per cent, are positive. In the material of Doctor Willier and Miss Rawles, thyroid is found in 60 per cent or 80 per cent of cases on the right; in 100 per cent of the left pieces. My primitive-streak material gave one positive case out of eleven right pieces (9 per cent); as contrasted with three positive out of twelve cases for the left side (25 per cent).

The temptation to ascribe great significance to these differences is one which I resist. It must be remembered that graft material is exceedingly variable; and that also it is possible that in many of these cases all of the presumptive thyroid material was included in the median rather than in the right piece. Nevertheless, the fact that the differences in

thyroid yield all lie in favor of the left side, is most interesting. If this difference is borne out in subsequent work, an interpretation must be offered ascribing superior developmental capacities in this particular, and perhaps in others, to the left side.

d. Histological analysis

In the preceding analysis, thyroid has been mentioned as present or not present in the grafts; the criteria of its identification have not been explained, nor other histological features dealt with. Such is the purpose of this section.

Thyroid in these grafts occurs in irregular, often lobed masses, distinct from other tissues (fig. 7). The length of such masses varies from 50 to 750 μ in my material; typical masses are 150 to 200 μ long. Histologically, thyroid appears as a body of cells of epithelial nature, arranged in cords of distinctive character. It is bounded by a capsule of thin fibrous mesenchyme. Capillaries containing erythrocytes are present between the cords (fig. 4). In further differentiated cases, follicles are seen. Eosinophile colloid may or may not be present in the follicles (fig. 8). These structures are not always compactly arranged, as in the normal thyroid, but may be separated from one another by mesenchyme. In some cases, the follicles are distended, apparently by the colloid within, so that they reach relatively enormous proportions. Any single thyroid mass may contain cords as well as follicles of all sizes. These structures undoubtedly represent stages in the formation of the definitive functional follicle, described by Bradway ('29).

In every series of grafts, cases occur where thyroid is poorly differentiated, with only a few typical regions, or even not identifiable with certainty. Such cases occur no more frequently in the primitive-streak material than in that from the head-process stage, as far as my data go.

The capillaries within the thyroid are seen in favorable cases to connect with the blood vessels of the graft, which, as far as is known, belong to the host circulatory system.

Frequently, though not invariably, ganglion cells lie contiguous or very near to the thyroid (fig. 6). These undoubtedly represent the large ganglion found next to the thyroid in the ten-day chick embryo.

The above description holds true for the thyroid obtained in grafts of the primordium itself as well as in transplants of early blastoderms. There is, however, a difference in appearance. In grafts from the seventy-two-hour stage, thyroid appears typically more compact, more heavily staining, more sharply circumscribed, with shape frequently very like the normal thyroid, than in the other material; although the more diffuse type is occasionally found. The association with gut may or may not occur. One's impression is that one is looking at an organ rather than at a mass of tissue.

As has already been indicated, thyroid in the head-process and primitive-streak material is always associated with entodermal tubes of one sort or another. A kind frequently found is composed of simple flat epithelium, without surrounding muscle (fig. 8). Such tubes are identified as pharynx. Often, however, thyroid is associated with a complex structure in which skin goes over to lower stratified epithelium, and finally to gut (shown in fig. 4). Such structures are interpreted as oral cavity. Thyroid, when present in connection with such a complex, is invariably seen to be most closely associated with flat epithelium: either with the simple type or with a low two-layered epithelium as in figure 4, like that found in the posterior oral cavity and parts of the pharyngeal region of the normal ten-day chick. It may also be mentioned that in some cases only the merest trace of gut is present.

The stage of differentiation of the thyroid found in grafts, as compared with normal thyroid of corresponding age, presents a striking situation. The total age of my grafts (age of donor plus time on the host membrane) runs from eight and one-half to twelve days. There is no discernible relation between the age of the graft and the degree of differentiation of the thyroid: well-differentiated thyroid with colloid,

approximating that found in the twelve- or thirteen-day chick, is present in grafts whose age totals eight and one-half days; in others, twelve days old, only cords are found, as in the ten-day normal chick. (Eosinophile colloid is not appreciably present before the eleventh day.) Compare figures 3, 4, 8. Attempts to correlate the degree of differentiation of thyroid with the presence or absence, either in its vicinity or in the whole graft, of any one structure, such as heart, liver, or nervous tissue, fail utterly. Likewise, the part of the blastoderm from which the graft arose seems not to be a factor: precocious thyroid may appear in any type of graft; the same is true of the less-differentiated sort. An exception appears to be the four median grafts from the head-process stage, which show no appreciable time displacement in this respect; however, in none of these cases can the thyroid be called well differentiated, so that the exception is hardly a convincing one.

Certain generalizations as to the relation of age of donor to the degree of differentiation of thyroid may perhaps be of interest. In the primitive-streak material, marked acceleration is frequent; in that from the head-process blastoderms, acceleration is less frequent and less striking. In grafts from three-day donors, no acceleration is observed; but marked retardation in some cases. In all this material, any one graft may contain, in close proximity, thyroid of widely differing degree of development.

Tissues other than thyroid, in grafts, show either development comparable to that in embryos of corresponding age, or else retardation. Thus, thyroid arising from the grafted primordium does not differ in this respect from other tissues. This effect as a result of the operation is completely understandable.

Potential thyroid in the definitive primitive-streak and to a less degree in the head-process stage has, then, the unique capacity of differentiating more rapidly than surrounding tissues, more rapidly than the normal control. This extreme lability of differentiation rate in embryonic thyroid under

conditions of the graft seems less astonishing when we consider the potentialities for growth and regression retained even by the adult thyroid. It is plain that the thyroid is an extremely sensitive system. The acceleration observed in grafts from early stages might very reasonably be attributed to hormone effects from the host; however, since we have seen that thyroid masses in the same graft (fig. 5) may be in very unequal stages of differentiation, a local differential must be thought of as operative in preventing acceleration in certain cases.

The indication of decreased capacity for accelerated differentiation with increased age of donor may, if we accept the present data as adequate—always a question with so variable material—suggest another property of preprimordial thyroid: in addition to being, as far as can be told, more strictly localized in the head-process stage than in the definitive primitive streak, it has less capacity for time displacement in the former stage: i.e., temporal as well as spatial delimitation has advanced.

GENERAL DISCUSSION

The foregoing experimental analysis, demonstrating capacity on the part of lateral pieces of the flat blastoderm to produce thyroid, has thus far only implicitly been connected with the course of normal development. The significance of the experimental results is perhaps so obvious as to merit formulation.

That patron saint of all good embryologists, Wilhelm His, in his beautifully simplified analysis of morphogenesis ('74), pointed out that the flat, undifferentiated blastoderm might be considered as an aggregate of organ-forming areas: that is to say, for every region of the adult body there is a corresponding area in the blastoderm, which will realize its potentiality through the mechanics of development: differential growth and the folding and stretching pursuant thereon. In view of the manner in which the chick embryo is formed, a map of the organ-forming germinal areas of an early blas-

totoderm would show dorsal organs medially located; ventral organs on either side. Thus a median ventral structure must of necessity have a bilateral origin.

According to the accounts of all authors since Remak ('55), the thyroid in the chick arises as a median ventral diverticulum of the pharynx, appearing after the ventral closure of the foregut at the level of the second pair of branchial clefts. The germinal areas corresponding to it must then at the primitive-streak and head-process stages be located laterally in the entoderm.

A concept that must be used to supplement His' scheme—one of which His himself was innocent—is that of the specificity of certain germinal areas: i.e., their capacity for differentiation independently of the rest of the organism. We may then regard the head-process blastoderm (to take a stage where the experimental results have been decisive) as a system where the main structures of the embryo-to-be are localized with reference to the mediolateral axis, and, if our data will not permit us to say segregated, are at least possessed of a degree of specificity as to their prospective potencies.

The work of Hunt ('31 a) on the primitive-streak and head-process stages of the chick, in conjunction with that of Willier and Rawles ('31 a), coincides very well with this sort of interpretation. Hunt has observed with regard to anteroposterior localization that axial structures arise only from the node level at the definitive primitive-streak stage; from the node and all levels anterior thereto after the head process has appeared. Working farther with the node at the stage of the definitive primitive streak, he has found that the node region is by no means an isotropic system, but that as far as can be told it has mosaic properties as regards the anteroposterior axis ('31 b). The levels I have worked with have all been anterior ones; the results obtained as to the mediolateral axis at these levels also tend to indicate at least a degree of localization in that region of the blastoderm from which the embryonic axis is to arise.

An important clarification of the mode of origin of lateral organs has been offered by Adelmann ('29 a, b; '30) in his work on eye potencies in the urodele neurula. Eye material at this stage he finds present as a single equipotential area across the anterior end of the neural plate; i.e., medially as well as laterally. This is the distribution that has been indicated for thyroid in the definitive primitive-streak stage. Whether the parallel is a true one, that is to say, whether the thyroid field is a single one at the stage mentioned, as Adelmann found the eye originally to be, or whether a median strip, however narrow, is present that does not possess thyroid potencies, remains yet to be seen. The suggested analogy is, of course, to be thought of only in the most general terms: the special factors concerned with the origin of eye from the ectoderm in the urodele on one hand, and those involved in the origin of thyroid from the entoderm of the chick on the other, must of necessity be very different.

The recent work of Marcus ('30, '31 a, b) indicates that in the amphibia, material from the appropriate region is capable of self-differentiation as thyroid as early as in the open medullary-fold stage. This study would form an interesting and close parallel to the present data on chick thyroid, were it not for the very unexpected morphological origin of the amphibian thyroid described by Marcus; i.e., by inward migration of the lower or sensory layer of ectoderm. This heresy must be confirmed or disproved before it can be profitable to make comparisons between amphibia and other groups in regard to thyroid development. For the chick, I may say that I have been unable to find figures or sections that could possibly be interpreted as indicating other than entodermal origin of thyroid in normal development. In my experimental material, it has not been possible to delimit ectoderm from entoderm in the oral-cavity-gut complexes with which thyroid is frequently associated; hence evidence from this angle is lacking.

SUMMARY

1. Thyroid differentiation and the localization of thyroid-forming potencies in the chick embryo were studied by means of the chorio-allantoic graft.

2. Right, median, and left pieces of the anterior end of the pellucid area of definitive primitive-streak and head-process blastoderms were studied with reference to their capacity for producing thyroid when grafted. Grafts of the thyroid of the seventy-two-hour chick were also observed.

3. Thyroid is found in all three types of graft from the primitive-streak and early head-process stages; after formation of the head fold, in lateral pieces only. This may indicate, *a*) the gradual lateral separation of two distinct centers, originally near the median line; or, *b*) the progressive bilateral localization of what was in the beginning a single broad field.

4. Although these grafts are not apt for demonstrating segregation, a degree of specificity of the prospective thyroid material at these preprimordial stages is indicated. At the seventy-two-hour stage the thyroid anlage is unequivocally segregated.

5. Thyroid in grafts from the definitive primitive-streak stage shows a remarkable capacity for differentiation strikingly accelerated as compared with normal thyroid of like age.

6. This capacity for acceleration decreases in the head-process stage, and has entirely disappeared by the seventy-two-hour stage. A fixation of potencies in this respect, as well as spatial localization, is indicated.

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PLATE 1

EXPLANATION OF FIGURES

Figures 3 to 8 are photomicrographs of preparations fixed in Bouin, sectioned 8μ , stained in Heidenhain's iron haematoxylin.

3 Section of normal thyroid of ten-day chick. $\times 433$.

4 High-power view of *a* in figure 5. Note large blood vessel entering at the right; capillaries between cords; oral-cavity-pharyngeal tube, upper left. Compare with figure 3. $\times 433$.

5 General view of part of a section of graft 84-5-B (DPS, median strip; total age, ten and one-half days), showing typical relation of heart, liver, thyroid, and oral cavity. *a*) thyroid consisting of cords; *b*) thyroid lobe with follicles and colloid; oral cavity to the left. *l*, liver; *h*, heart. $\times 75$.

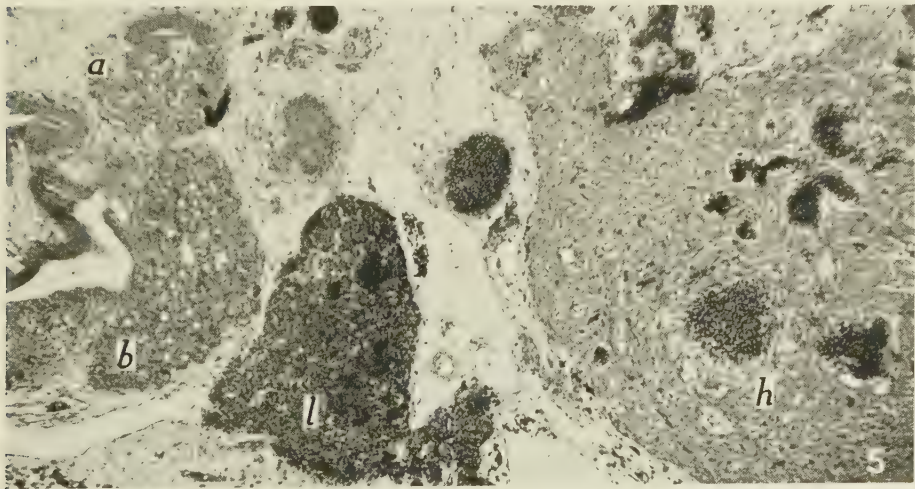
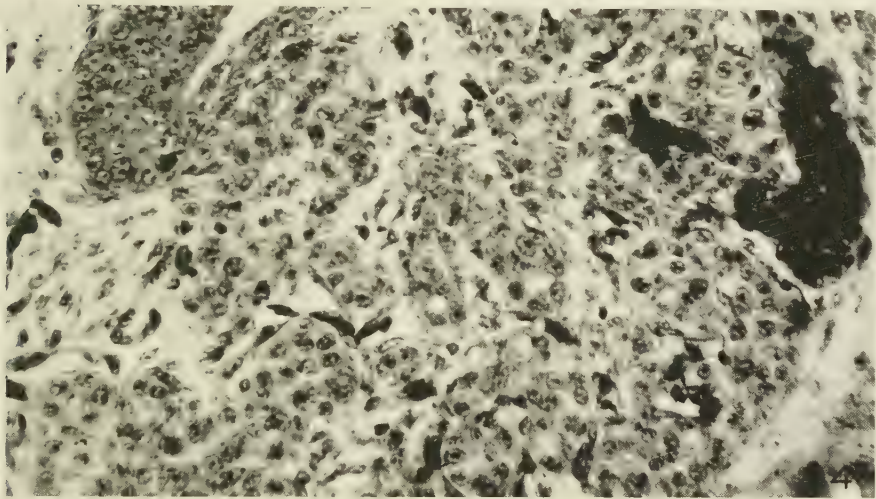
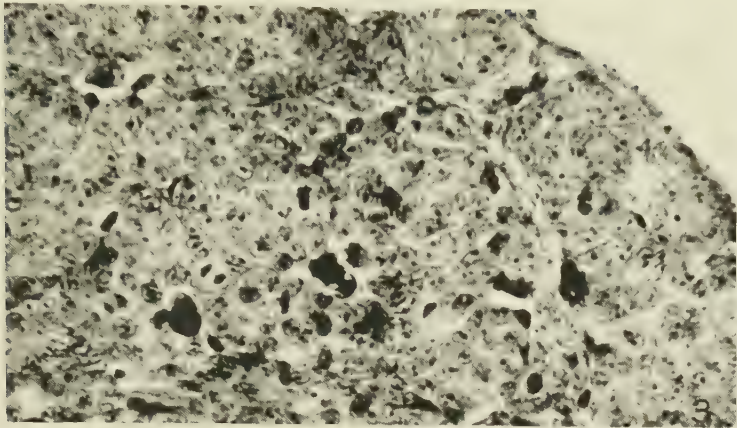


PLATE 2

EXPLANATION OF FIGURES

6 Thyroid in graft 68-7 (*HP*, right piece; total age, nine days). Note ganglion cells (*g*) at edge of mass. $\times 400$.

7 Thyroid (*t*) in graft 94-2-C (left piece, *DPS*+; total age, eight and one-half days). Note histological specificity notwithstanding close association with liver (*l*) below. The dark nuclei of the liver are distinctive. $\times 400$.

8 Thyroid in graft 93-3-B (median piece, *DPS*; total age, nine and one-half days). Note well-formed follicles with colloid; pharyngeal tube (*p*) at the right. Compare with figures 3 and 4 as to stage. $\times 400$.

